

Treatment of sewage and kitchen waste for recovery of methane and biomass and its reuse in fish culture

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ABSTRACT

Performance of the laboratory-scale wastewater treatment system, consisting of up-flow anaerobic sludge blanket (UASB) reactor, followed by an algal tank and a duckweed tank in series, was investigated for treatment of sewage and kitchen waste, to evaluate suitability of treated effluent for fish culture and recovery of biogas and biomass. The UASB reactor was operated at different organic loading rates (OLRs) ranging from 0.13 to 3.79 kg COD/m³ d. The UASB reactor demonstrated maximum chemical oxygen demand (COD) and biochemical oxygen demand (BOD) removal efficiency of 88 and 90%, respectively, when operated at an OLR of 2.93 kg COD/m³ d. The effluent from the UASB reactor was post-treated in an algal (*Spirulina platensis*) tank for efficient nutrient removal. The highest NH₄⁺-N and PO₄³⁻ removal efficiency of 75.8±6 and 80.4±7 %, respectively, was observed in algal tank. However, the effluent BOD and total suspended solids (TSS) were relatively high, due to the presence of algae. Passing of algal tank effluent through a duckweed (*Lemna gibba*) tank was found to remove algae due to reduced light penetration and refining the water quality. The highest NH₄⁺-N and PO₄³⁻ removal efficiency of 46.9±14 % and 78.7±8 %, respectively, was observed in duckweed tank. In both algal and duckweed tank, NH₄⁺-N and PO₄³⁻ removal efficiency decreased with increase in OLR.

The treated effluent from duckweed tank was admitted into the fish culture tank where tilapia fish were cultured. All the tilapia fish survived in the treated water throughout the experimental period of ten months. The highest dry matter yield of 63.27 and 32.25 kg/ha.d of *S. platensis* and *L. gibba*, respectively, were obtained. Algae and duckweed biomass produced during this treatment process have revenue generating potential since this biomass can be used as feed for cattle, poultry and fishes. This integrated treatment system demonstrated that production of biogas, algae and duckweed biomass and treated wastewater for reuse in fish culture can be achieved in this simple system.

Key words: UASB reactor, Kitchen waste, Sewage, Biomass, Biogas, *Spirulina platensis*, *Lemna gibba*, Tilapia fish.

INTRODUCTION

Wastewater comprises liquid waste discharged by domestic residences, commercial properties, industry and/or agriculture and can encompass a wide range of potential contaminants, ranging from nutrients to toxic heavy metals. Kitchen waste or food waste typically consists of vegetable peels, meat scraps, excess or spoiled prepared food and other discards, along with the utensils wash water from domestic or commercial kitchens.

This waste stream, when discharged into the nearby water bodies or dumped in open landfills, can cause serious environmental problems. Kitchen wastes contain high concentration of biodegradable organic compounds. If suitable treatment technologies are employed, then valuable byproducts can be recovered from this organically rich waste.

Anaerobic treatment of wastewater in Up-flow Anaerobic Sludge Blanket (UASB) reactor is an attractive process, because it recovers methane gas during efficient treatment of wastewater. Several

studies carried out in both pilot-scale and full-scale systems have demonstrated that the UASB reactor is a reliable and simple technology for treatment of domestic wastewater [1, 2].

However, the UASB effluent still contains high amount of nutrients and counts of faecal microorganisms. Discharge of this nutrient rich inorganic and organic compounds from UASB reactor cause eutrophication of receiving water.

Furthermore, this UASB effluent still has polluting effects due to their high nitrogen, phosphorous and suspended solid concentration, and may be due to higher values of biochemical oxygen demand (BOD) and chemical oxygen demand (COD) in some cases. In order to achieve the effluent quality as stipulated by the World Health Organization for unrestricted irrigation, the UASB effluent should undergo post-treatment [3].

Based on the performance reported in literature for the UASB reactor, it is evident that the post treatment of UASB effluent is necessary, if the effluent is intended

to be reused for certain application or discharged into the nearby stream. The post-treatment may be required for the removal of total suspended solids (TSS), nutrients and pathogens. The nutrient-rich effluent from the UASB should be post-treated, not to remove nutrients but to recover these nutrients.

As a post-treatment alternative, wastewater treatment involving micro-algae and duckweed is quite attractive because of its capacity to transform waste into useful biomass using sunlight as an energy source. Wastewater can then be viewed as a valuable substrate for the production of potentially useful biological compounds.

The use of hyper-concentrated algal culture is potentially interesting because it shortens the time required for nitrogen and phosphorus removal from days to a few hours. *Azolla*, *Chlorella* and *Spirulina* are important algae which can effectively purify wastewater. High rate algae ponds represent one of the most popular treatment methods and provide a huge amount of valuable algae [4]. Pond fish production tied with algal culture is also very effective in wastewater treatment.

A drawback of shallow algal pond is the low efficiency for TSS and BOD removal, due to the presence of algae in the effluent [5]. This could result in difficulties to satisfy discharge criteria for BOD or in reuse applications for drip-irrigation or aquaculture. These algae can be removed from effluent by passing through a stage with reduced illumination. This shading is expected to cause the algae to die and to settle or disintegrate. The shading can be done in a duckweed pond (DP).

Duckweeds are very small floating aquatic macrophytes belonging to the family of *Lemnaceae*. The high growth rate of this macrophyte permits regular harvesting of the biomass and hence nutrients are removed from the system. The produced biomass has an economic value because it can be applied as fodder for poultry and fishes [6-8]. The duckweed production and nutrient removal potential of the system are reported by Van der Steen *et al.* [9].

The possible reuse of treated wastewater includes non-potable uses such as agriculture, park irrigation, road side horticulture, aquaculture and fire-fighting and in-house uses such as flushing toilets and urinals, primarily in commercial or office buildings. If treatment systems are selected properly, the wastewater can be considered as a resource to recover valuable products and to get treated water meeting the desired reuse quality criterion. Such practice will make

overall operation of the wastewater treatment sustainable.

Hence, the main objectives of this study were: (1) to investigate the performance of UASB reactor integrated with algal tank and duckweed tank in treating wastewater under various hydraulic loading conditions, and the reuse of treated water for fish culture; and (2) to quantify the production of algae and duckweed.

MATERIALS AND METHODS

Sewage generated from the Indian Institute of Technology, Kharagpur campus and kitchen waste generated from the institute staff canteen were used for the study. The kitchen waste collected was a mixture of leftover or stale, cooked and uncooked food, extracted tea powder, waste milk and milk products, etc. The vegetables refuse like peels of various vegetables, rotten potatoes, and tomatoes, coriander leaves, etc. were also present in the waste.

The collected waste was crushed and mixed with water (about 3 % solids concentration w/w) by means of a mixer for four to five days and used as a feed to the UASB reactor. During vacation period, when kitchen waste was not available, sewage collected every day from the sewage pumping station was used as feed to UASB reactor.

The lab-scale experimental set-up (Fig 1) used in the study consists of a UASB reactor fed continuously by means of a peristaltic pump (MICLINS-PP-20-4C). The effluent from the UASB reactor was fed into an algal tank, then into a duckweed tank and finally into a fish culture tank by gravity. All experiments were conducted at ambient room temperature varying from 18 to 32 °C.

Up-flow Anaerobic Sludge Blanket (UASB) Reactor

The collected domestic wastewater or prepared kitchen wastewater was pre-treated in a UASB reactor (total working volume of 12.57 L) for the removal of organic matter. The UASB reactor was made up of acrylic cylinder of 10 cm internal diameter and 160 cm effective height. Gas-liquid-solid separator of 18.5 cm height was provided at the top of the UASB reactor.

Algal Tank (Post-treatment)

Effluent from the UASB reactor was passed through a tank of dimension: length 60 cm, width 30 cm and depth 30 cm in which a hyper concentration of algae was maintained. Algae species selected for this study was *Spirulina platensis*.

Purified stock culture of *S. platensis* was obtained from the Biotechnology Laboratory, Indian Agricultural Research Institute (IARI), New Delhi, India. The culture was grown in 500 mL culture flasks using BG-11 as a media [10]. Isolates were maintained at an optimum temperature of 30°C. Illumination was provided with white fluorescent tubes, giving a light intensity of 2500 lux (measured by a digital Lux meter KM-LUX-99) and a photoperiod of 12 h light and 12 h

darkness was adopted. The culture was kept in suspension by placing the culture flask on a shaker. The culture was used for inoculation when it reached the exponential growth phase. The cells were filtered, washed and inoculated at a starting concentration of approximately 0.2 g/L (dry weight). During the experimental period a light intensity of 2000 lux unit was maintained during day time.

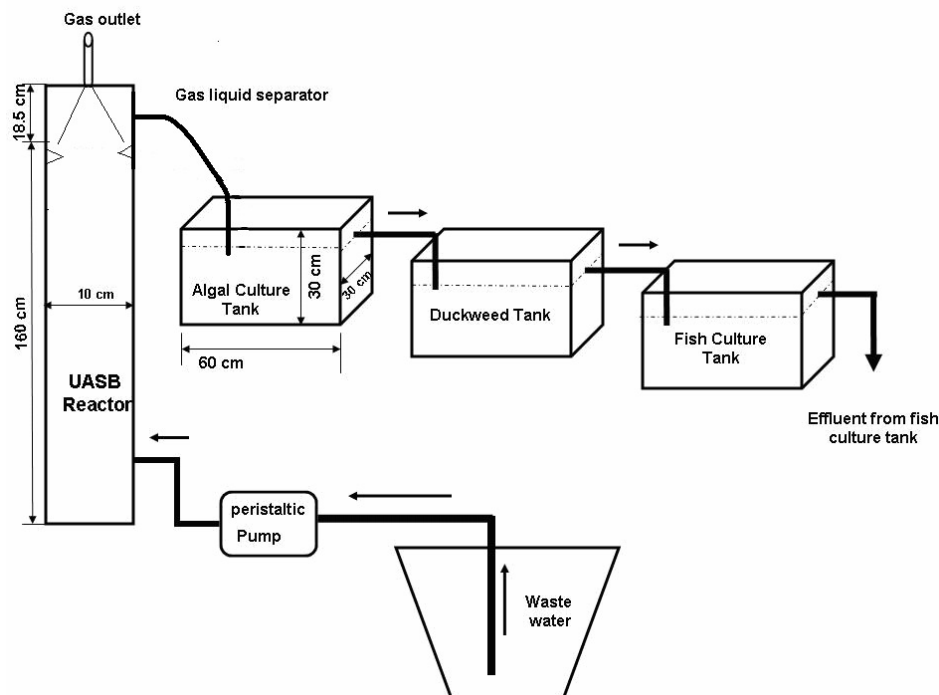


Fig. 1 Line diagram of the experimental set-up showing UASB reactor, algal tank, duckweed tank and fish culture tank arranged in series. The duckweed tank and fish culture tank were of the same dimensions as shown for algal culture tank.

Duckweed Tank (Post-treatment)

Treated effluent from algal tank was passed through a duckweed tank of length 60 cm, width 30 cm and depth 30 cm. The duckweed species selected for the present study was *Lemna gibba*. The sample of *L. gibba* used in the experiment was collected from nearby pond (Kharagpur).

Duck weed, which was also cultivated in the controlled environment, also received the same amount of light as in the algal tank (2000 lux unit) during the experiments. All the experiments were started with 55 gm fresh weight of *L. gibba*.

Reuse of Treated Effluent for Fish Culture

Treated effluent from the duckweed tank was passed to the maturation tank of length 60 cm, width 30 cm and depth 30 cm, where Tilapia fish were grown. Four numbers of fishes were released into the fish tank, with an average initial body weight of 13.817 gm, initial length of 9.2 cm and body width of 3.75 cm. The fish were fed with pellet diet containing 35 % crude protein and 12 % fat.

Water Quality Analysis

The collection of representative water samples is an integral part of the total analytical procedure, so all efforts were put for getting a correct picture of water quality. The well-mixed water samples were collected from the feed tank for establishing characteristics of wastewater. Grab samples were collected from the effluent pipe of UASB reactor to ascertain the performance of the UASB reactor and to understand influent water characteristics to the algal tank. Similarly, water samples were collected from the effluent pipe of algal tank and duckweed tank to ascertain the performance of algal tank and duckweed tank. Water quality parameters in the fish tank were also measured at regular intervals. The pH was measured by using digital pH meter (SYSTRONICS - 802) on daily basis.

Dissolved oxygen (DO) and temperature were measured by using a DO meter (YSI-MODEL - 55) on daily basis. The COD was determined at two days interval. BOD was determined on every fourth day. Ammonia nitrogen ($\text{NH}_4^+\text{-N}$), nitrite nitrogen (NO_2^- -

N), nitrate nitrogen (NO_3^- -N), orthophosphate (PO_4^{3-}) and TSS were measured on every three days interval. The procedures followed for all these parameters were as per the Standard Methods [11].

Growth Rate of Algal Biomass

For determining algal biomass growth, 100 ml of well mixed water sample was collected from the culture tank, filtered through a pre-dried and pre-weighed glass filter paper (Whatman GF-C) and dried at 60°C for 24 h. Biomass growth rate k (day^{-1}), was calculated by using the following equation [12]:

$$k = \frac{W_2 - W_1}{W_1 \times t} \quad (\text{Eqn. 1})$$

Where, W_1 = initial weight; W_2 = final weight; and t = days of culture.

Growth Rate of Duckweed

All the experiments were commenced with 55 gm fresh weight of *L. gibba*. Fresh weight of duckweed was determined after rinsing and blotting on tissue paper. Sub-samples were dried at 60°C for determination of the initial dry weight (DW_0). This resulted in a density of approximately 16 g DW m^{-2} covering approximately 60 % of the tank surface. This density was chosen to prevent extensive algal growth as well as reduced growth of duckweed through overcrowding. Duckweed biomass was harvested at the end of the experiment, rinsed and sub-sample was dried and weighed (DW_1) and relative growth rates (RGR) (day^{-1}) were calculated from the dry weights by using the following equation [13]:

$$\text{RGR} = \frac{\ln \text{DW}_1 - \ln \text{DW}_0}{t} \quad (\text{Equ. 2})$$

Where, t = days of culture.

Specific Growth Rate of Fish

Four numbers of fish were released into the fish tank. The weights of fish were measured at the beginning and end of each experiment. Specific Growth Rate (SGR) was calculated by using the following formula:

$$\text{SGR} = \frac{\ln W_2 - \ln W_1}{D} \quad (\text{Equ. 3})$$

Where, W_1 = initial weight; W_2 = final weight; and D = days of culture.

Protein and Lipid Content of *S. platensis* and *L. gibba*

Protein content of the cultured algae and duckweed was determined by “Kjeldahl Equipment”, whereas,

the lipid content was determined by “Soxtherm Equipment”.

RESULTS AND DISCUSSION

Performance of the UASB Reactor

Feasibility studies on organic matter removal and methane recovery from the waste was carried out by using five different hydraulic retention times (HRTs) in the UASB reactor. At the beginning, the UASB reactor was fed with sewage (Experiment no. 1) and afterwards with kitchen waste having about 3% solid concentration.

The kitchen waste, which was collected in solid form, was mixed with the required amount of tap water by using a mechanical mixer to increase solubilization and the supernatant was pumped in the UASB reactor. Overall there was no substantial variation in pH in all the experiments, whereas temperature was recorded to be low during the month of December and January. However, no adverse effect of the temperature on reactor performance was noticed.

Expt. 1 was carried out with low strength sewage with an average COD of 158 mg/L and BOD of 78 mg/L and the remaining experiments, from Expt. 2 to Expt. 5, were carried out with high strength kitchen waste. The ratio of COD and BOD values varied between 1:0.49 and 1:0.61. These values are lower than the value (1:0.67) observed in easily biodegradable organic wastewater [14].

The UASB reactor, employed as the first stage treatment process, was operated under different organic loading rates (OLRs) between 0.126 and 3.79 kg COD/ m^3d , and HRT between 30.12 h and 13.38 h. The range of COD and BOD removal efficiencies varied between 69.3 ± 4.8 and 88.3 ± 1.4 % and 72.4 ± 6.8 and 90.4 ± 2.4 %, respectively (Table 1). This range is in agreement with the earlier reported values [15].

During Expt. 1, when the UASB reactor was operated at OLR of 0.126 kg COD/ m^3d and HRT of 30.12 h, COD and BOD removal efficiency were minimum as 69.3 ± 4.8 and 72.4 ± 6.8 %, respectively. After increasing the OLR and reducing the HRT, it was observed that COD and BOD removal efficiency of the reactor increased.

The Expt. 4 showed the highest COD and BOD removal efficiency of 88.3 ± 1.4 and 90.4 ± 2.4 %, respectively. The high COD and BOD removal efficiency was comparable to those reported in the literature by Mario *et al.* [16]. These results enunciate, that within the OLR range studied in these

experiments, with increasing OLR and reducing HRT the efficiency of reactor increases in terms of COD and BOD removal. Mario *et al.* also had similar observation [16].

The wastewater used as a feed to the UASB reactor was having TSS in the range of 84 to 158 mg/L. The effluent TSS value varied between 21.9 and 41.5 mg/L, exhibiting TSS removal efficiency between 73.4 and 77.6 %. Relatively low treatment efficiencies are expected for nitrogen and phosphorous removal in UASB reactor; since anaerobic reactors are known to remove negligible amount of nutrients.

Experiment 2, 3, and 4 showed slight removal of $\text{NH}_4^+\text{-N}$ in the UASB reactor, which could be due to both new biomass production, as well as precipitation in the reactor. Whereas, $\text{NH}_4^+\text{-N}$ concentration increased during Expt. 1 and 5, perhaps due to anaerobic bioconversion of proteins in the wastewater into amino acids and then to ammonia [17].

The phosphate of the feed varied between 1.25 and 3.58 mg/L, respectively. The anaerobic process is a mineralization process, so only small amounts of PO_4^{3-} was removed. The PO_4^{3-} removal efficiency varied between 40.7 and 48.1 %, respectively.

These values are higher than the result reported by EL-Shafai *et al.* [18]. Amongst the five experiments, the highest PO_4^{3-} removal of 48.1% was observed in the Expt. 2. Higher PO_4^{3-} removal observed in the experiments could be addressed to the precipitation and assimilation of phosphorous in the reactor bacterial biomass [17].

Biogas Production in UASB Reactor

Range of biogas production rate which was observed under different experimental runs is shown in Table 2. Under different OLRs, the average gas production varied between 0.29 ± 0.14 and 14.35 ± 5.5 L/d. The highest biogas production rate of 8.6 to 24.21 L/d and an average of 14.35 ± 5.5 L/d was observed in Expt. 5 operated at higher OLR of $3.79 \text{ kg COD/m}^3 \text{ d}$.

In the range of OLR applied to the reactor in this experiment, higher biogas yield was observed when higher OLR is used, as reported earlier [19]. The gas yield, defined as the amount of gas produced from a given quantity of organic matter removed as a result of the activity of the anaerobic microorganisms, varied between 0.26 and $0.36 \text{ m}^3/\text{kg COD removed}$.

In this study, the highest gas yields of $0.36 \text{ m}^3/\text{kg COD removed}$ was observed in Expt. 5, and it was similar to that reported by Parawira *et al.* for treating potato

waste leachate in an UASB reactor at an OLR of $4.7 \text{ kg COD/m}^3 \text{ d}$ [20].

Performance of Algal Tank

The nutrient rich effluent from UASB reactor after substantial removal of organic matter was treated in an algal tank, with *S. platensis* as the algal species. Nutrients are trapped by the aquatic plants to produce protein rich biomass. The average water quality observed in the effluent of algal tank is presented in the Table 3.

Monitoring of performance the algal tank during the experiments revealed that the total efficiency of COD and BOD was not significantly high as compared to UASB reactor. The COD and BOD removal efficiencies were in the range of 38.2 ± 10.8 to 63.8 ± 7.4 % and 20.8 ± 9.1 to 60.6 ± 4.4 %, respectively. The highest COD removal efficiency of 63.8 % was observed in the Expt. 2, operated at lowest OLR during treatment of kitchen waste.

The effluent TSS values in the algal tank varied between 15 and 28 mg/L. TSS removal efficiency varied between 31 ± 6 and 41 ± 16 %, respectively. This value was considered, lower than the reported by Patnaik *et al.* in batch cultivation of *S. platensis* for the treatment of raw sewage, at HRT of 8 days [21].

The $\text{NH}_4^+\text{-N}$ and PO_4^{3-} in the feed varied between 4.98 and 8.18 mg/L and between 0.74 and 2.07 mg/L, respectively. The observed $\text{NH}_4^+\text{-N}$ and PO_4^{3-} removal efficiencies were in the range of 60.8 ± 3.1 to 75.8 ± 6.6 % and 70.3 ± 4.6 to 80.4 ± 6.8 %, respectively. This efficiency was little lower than the reported value by Patnaik *et al.* [21]. Expt. 1 showed the highest $\text{NH}_4^+\text{-N}$ and PO_4^{3-} removal efficiency with 75.8 ± 6.5 and 80.4 ± 6.8 %, respectively.

The growth rate (GR) of *S. platensis* at different HRT is summarized in Fig. 2. The GR ranged between 0.07 and 0.12 d^{-1} . The highest GR of 0.12 d^{-1} was observed in Expt. 1 operated at HRT of 120 h. This value agrees with the biomass growth rate of 0.11 d^{-1} for *S. platensis* cultured on fish cultured effluent, reported by Kamilya *et al.* [12]; and it was higher than the growth rate reported by Lodi *et al.* [22] for *S. platensis* cultured in the inorganic nutrient media.

The GR was mainly affected by the environmental parameters. Expt. 1 was conducted during the month of October when the average water temperature was 28.5°C , which is favorable for the *S. platensis* growth. The low growth rate observed in the Expt. 2 and 3 was attributed, to the lower temperature during these experiments.

Performance of Duckweed Tank

The effluent from algal tank was post-treated in aquatic macrophyte-based treatment system with duckweed species *Lemna gibba*. Duckweed has high growth rate, due to which it further refine the effluent water quality. Performance of duckweed tank is presented in Table 4. The COD of the feed varied between 29 and 101 mg/L, and the BOD of the feed varied between 16 and 50 mg/L.

The observed average COD and BOD removal efficiencies were in the range of 27.9 ± 8.4 to 64.4 ± 7.8 % and 32.1 ± 9.4 to 69.4 ± 8.2 %, respectively. The maximum COD and BOD removal efficiency of 64.4 ± 7.8 and 69.4 ± 8.2 %, respectively, was observed in the Expt. 2.

These values are in agreement with results obtained by EL-Shafai *et al.* while treating effluent of the UASB reactor in the duckweed ponds [18]. They reported removal efficiency of 64% and 73% during the warm season for COD and BOD removal, respectively.

BOD and COD removal rates between 50 and 95% have been reported for duckweed-covered systems [23]. It was observed that, with increase in OLR beyond $0.0054 \text{ kg COD/m}^2\text{.d}$ and decrease in HRT below 96 h, the COD and BOD removal efficiency in the duckweed tank decreases.

The effluent TSS value in the duckweed tank varied between 6.5 and 13.8 mg/L. The TSS removal efficiency ranged between 48.4 ± 4.3 and 58.7 ± 3.9 %. This is similar to that reported by EL-Shafai *et al.* [18]; where they reported an efficiency of 43 to 63%, at a HRT of 15 days. The highest TSS removal efficiency of 58.7 ± 3.9 % was observed in the Expt. 3, operated at HRT of 72.73 h.

The $\text{NH}_4^+\text{-N}$ and PO_4^{3-} of the feed was in the range of 1.21 to 3.02 mg/L and 0.14 to 0.61 mg/L. The $\text{NH}_4^+\text{-N}$ and PO_4^{3-} removal efficiencies under different experiments were in the range of 42.7 ± 4.4 to 47.3 ± 8.6 % and 72.1 ± 8.0 to 78.6 ± 8.4 %, respectively. Expt. 1 showed the highest PO_4^{3-} removal efficiency of 78.6 %, with $\text{NH}_4^+\text{-N}$ removal of 47.1%. These values are less than that reported by Korner and Vermaat [24]. The highest nutrient removal efficiency was attributed to the highest growth rate and favorable temperature conditions during the Expt. 1.

The relative growth rate (RGR) of *L. gibba* in different experiments varied between 0.067 to 0.089 d^{-1} . This range was significantly lower than those measured by Korner and Vermaat [24]. Maximum value of RGR of 0.089 d^{-1} was observed in Expt. 1 operated at HRT of 120 h. Fig. 3 shows the variation in RGR of *L. gibba*

during different experiments. The reduction in RGR in Expt. 2, 3, 4, and 5 was mainly due to reduction in HRT and application of higher organic loading rates.

Reuse of Treated Wastewater for Fish Culture

Treated effluent from duckweed tank was admitted to the tilapia fish culture tank. A separate tank for fish culture was used, since the COD, BOD, TSS and nutrients concentration in the algal and duckweed tank were higher than the acceptable limit for fish culture. Moreover, DO concentration in the duckweed tank was less, due to surface shading.

The observed water quality parameters in the fish culture tank are summarized in the Table 5. Fish were fed with artificial pellet food (35% crude protein and 12% fat) during Expt no. 1 to 4 and harvested biomass during Expt. No. 5.

Specific Growth Rate of Fish

Four numbers of tilapia fish were released in the fish culture tank, and no mortality was observed throughout the experimental period. Tilapia fish thrived in the treated wastewater.

The SGR of fish in different experiments varied between 0.33 and 0.15 (% bw/d). During Expt. 1 to 4, the fish were fed with pellet feed; whereas, during Expt. 5, fish were fed with a mixture of harvested algae and duckweed (5 gm, 1:1). The fish specific growth rate was observed to be lower than the value reported by Gaigher *et al.* [25]. The highest SGR of 0.335 was observed in the Expt. 2.

Proximate Composition of *S. platensis*

Proximate analysis, the measurement of the amounts of moisture, protein, carbohydrate, and ash, was routinely conducted at the end of experimental runs. The proportion of the individual components varied to a certain extent due to the particular culture conditions.

The proximate composition of *S. platensis* in all the five sets of experiment is shown in Table 6. The protein and ash content varied between 59.73 and 63.25% and 6.21 and 8.46 %, respectively. These values are in agreement with the values reported by Becker [26] for *S. platensis* cultured in standard inorganic medium. Highest protein content of 63.25 % was observed in Expt. 1, and the lowest fat content of 6.21 % was also observed in Expt. 1.

Proximate Composition of *L. gibba*

The proximate composition of *L. gibba* in all the five sets of experiments is shown in Table 7. In this study, the dry matter content of the duckweed harvested ranged between 5.86 and 6.27 %. This range is slightly higher than that published in literature. The dry matter

content of *Lemna* species ranged between 5.55 and 5.35 % [6, 27, 28]. The protein content of the harvested duckweed ranged between 21.61 and 31.54 % on dry matter basis. Highest protein content of 31.54 % was observed in the Expt. 1, operated at an OLR of 0.0016 kg COD/m².d and HRT of 120 h. Culley and Epps reported a range of 14 to 25.9 % protein content in the dry matter of *L. gibba* grown on sewage [29]. Oron *et al.* reported a protein content of 15 to 48.1 % in dry matter of *L. gibba* grown on sewage [6].

Biomass Production in the Form of *S. platensis* and Duckweed

During the experiment the highest dry matter yield of 63.27 kg/ha.d was observed in the Expt.1. In the subsequent experiments, the *S. platensis* yield was observed to be 35.49, 56.94, 48.61 and 55.55 kg/ha.d. This was less than the reported value of 84.62 kg/ha.d by Mahadevaswamy and Venkataraman for *S.*

platensis cultured in effluent of biogas plant treating poultry droppings [30]. The harvested *S. platensis* can be used as a feed for animals and fishes. *S. platensis* contains the most remarkable concentration and combination of nutrients, antioxidants (like beta-carotene) and vitamins (vitamin E, A, B1, B5, B6 & B12) known in any food, plant grain or herb [31].

For *L. gibba*, the highest dry matter yield of 32.25 kg/ha.d was observed in the Expt.1. In the subsequent experiments, *L. gibba* yield was observed to be 27.31, 24.60, 26.68 and 28.03 kg/ha.d. The duckweed yield obtained in this experiment was less as compared to the yield reported in the literature as 126 to 138.8 kg/ha.d [18]. The lower yield of duckweed could be attributed to less nitrogen and phosphorous received in this tank because of substantial removal of these nutrients in the earlier algal tank. The harvested duckweed can be used as fodder for cattle, poultry and fishes.

Table 1: Performance of the UASB Reactor

Parameter	Expt. 1	Expt. 2	Expt. 3	Expt.4	Expt. 5
Flow rate (L/d)	10	12.5	16.5	18.5	22.5
HRT (h)	30.12	24.096	18.25	16.28	13.38
OLR (kg COD/m ³ .d)	0.126	1.384	2.354	2.925	3.794
Influent pH	7.37±0.36	7.14±0.19	7.32±0.15	7.24±0.19	7.28±0.16
Effluent pH	8.28±0.29	8.16±0.09	8.28±0.19	8.19±0.22	8.11±0.37
Influent temperature (°C)	27.7±2.5	23.3±1.4	19.9±1.5	18.3±0.9	25.1±1.7
Effluent temperature (°C)	27.7±1.1	23.0±1.6	21.4±1.27	19.4±0.4	26.9±2.2
Influent COD (mg/L)	158±15	1389±111	1790±253	1984±248	2116±231
Effluent COD (mg/L)	49±9	218±34	222±42	229±28	260±35
COD Removal Efficiency (%)	69.33±4.77	84.25±2.33	87.5±2.35	88.3±1.4	87.6±1.81
Influent BOD (mg/L)	78±12	737±94	898±54	1061±176	1284±228
Effluent BOD (mg/L)	21±5	100±16	97±11	99±15	125±55
BOD Removal Efficiency (%)	72.39±6.8	86.3±2.2	89.1±1.5	90.39±2.4	89.9±3.1
Influent TSS (mg/L)	84.2±8.0	124.1±12.1	134.2±13.4	150.5±12.1	158.2±20.5
Effluent TSS (mg/L)	21.9±3.8	27.6±2.8	33.4±5.9	37.5±5.9	41.5±6.9
TSS Removal Efficiency (%)	73.98±3.2	77.59±3.2	74.55±7.0	74.96±4.2	73.42±5.5
Influent NH ₄ ⁺ -N (mg/L)	4.66±0.35	7.73±0.65	8.51±0.64	7.84±0.61	7.68±0.25
Effluent NH ₄ ⁺ -N (mg/L)	4.98±0.23	7.64±0.65	8.18±0.49	7.77±0.64	7.7±0.17
Influent NO ₂ ⁻ -N (mg/L)	1.18±0.10	1.54±0.35	2.3±0.60	1.60±0.34	1.91±0.30
Effluent NO ₂ ⁻ -N (mg/L)	0.086±0.01	0.20±0.04	0.28±0.05	0.26±0.040	0.63±0.09
Influent NO ₃ ⁻ -N (mg/L)	1.48±1.20	3.031±0.35	3.40±0.49	3.24±0.48	2.94±0.32
Effluent NO ₃ ⁻ -N (mg/L)	1.04±0.21	2.08±0.21	2.44±0.26	2.35±0.23	3.26±0.47
Influent PO ₄ ³⁻ (mg/L)	1.25±0.19	2.76±0.21	3.40±0.29	3.45±0.16	3.58±0.23
Effluent PO ₄ ³⁻ (mg/L)	0.74±0.14	1.42±0.18	1.82±0.22	1.9±0.14	2.07±0.15
PO ₄ ³⁻ Removal Efficiency (%)	40.70±7.40	48.10±7.7	45.79±9.36	44.84±6.26	41.7±5.6

Expt. – Experiment number

Table 2: Biogas production rate in UASB reactor

	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
Bio gas (L/d)	1.6-3.2	3.6-4.8	6.1-15.9	7.1-18.65	8.6-24.21
Averaged (L/d)	0.29±0.14	4.3±1.2	8.8±3.1	10.9±4.1	14.35±5.5
Biogas production (m ³ /kg COD removed)	0.26	0.29	0.33	0.34	0.36

Table 3: Performance of algal tank

Parameter	Expt. 1	Expt. 2	Expt. 3	Expt.4	Expt. 5
Flow rate (L/d)	10	12.5	16.5	18.5	22.5
HRT (h)	120	96	72.73	64.86	53.33
OLR (kg COD/m ² .d)	0.0027	0.0152	0.0203	0.0235	0.0325
Effluent pH	8.47±0.18	8.35±0.26	8.48±0.08	8.49±0.21	8.28±0.21
Influent DO (mg/L)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Effluent DO (mg/L)	3.03±0.28	2.93±0.39	2.75±0.30	2.88±0.25	2.77±0.22
Effluent temperature (°C)	28.5±1.2	23.2±1.1	19.1±0.8	17.9±1.0	24.9±1.1
Effluent COD (mg/L)	29.4±3.7	77.7±11.9	84.9±15.9	83.1±5.6	101.0±21.4
Effluent BOD (mg/L)	16.4±1.9	43.1±10.6	42.5±4.0	38.6±2.8	49.9±10.7
Effluent TSS (mg/L)	15.0±1.9	16.06±3.3	21.40±3.6	24.76±3.4	28.32±3.1
Effluent NH ₄ ⁺ -N (mg/L)	1.21±0.37	2.45±0.53	2.79±0.34	2.79±0.19	3.02±0.27
Effluent NO ₂ ⁻ -N (mg/L)	0.18±0.02	0.30±0.04	0.33±0.05	0.32±0.034	0.68±0.09
Effluent NO ₃ ⁻ -N (mg/L)	1.29±0.19	3.14±0.31	3.4±0.42	3.34±0.13	3.44±0.43
Effluent PO ₄ ³⁻ (mg/L)	0.14±0.05	0.311±0.09	0.44±0.03	0.48±0.02	0.61±0.07

Note: Effluent of UASB reactor is admitted to the algal tank; hence, the value reported for UASB reactor effluent in the Table 1, for respective parameter, is the influent value for the algal tank for that parameter.

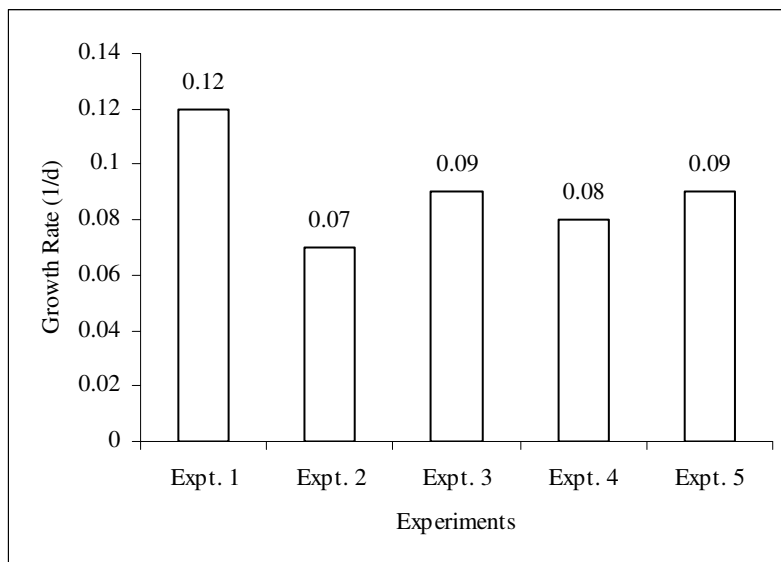


Fig. 2 Growth rate of *S. platensis* in different experiments

Table 4: Performance of duckweed tank

Parameter	Expt. 1	Expt. 2	Expt. 3	Expt.4	Expt. 5
Flow rate (L/d)	10	12.5	16.5	18.5	22.5
HRT (h)	120	96	72.73	64.86	53.33
OLR (kg COD/m ² .d)	0.0016	0.0054	0.0078	0.0086	0.0126
Effluent pH	8.33±0.26	8.50±0.20	8.69±0.08	8.47±0.22	8.30±0.10
Effluent DO (mg/L)	3.33±0.42	2.65±0.35	2.69±0.31	2.78±0.23	2.80±0.15
Effluent temperature (°C)	28.9±0.8	23.3±1.3	18.9±0.5	16.9±0.7	25.5±2.5
Effluent COD (mg/L)	21.0±2.7	27.3±5.8	34.7±7.2	37.8±7.1	50.6±12.3
Effluent BOD (mg/L)	11.1±1.40	12.6±1.9	16.1±2.6	15.5±6.7	22.9±3.8
Effluent TSS (mg/L)	7.7±1.1	6.5±0.9	8.8±1.2	10.4±1.5	13.8±1.7
Effluent NH ₄ ⁺ -N (mg/L)	0.64±0.25	1.29±0.22	1.55±0.14	1.58±0.106	1.73±0.13
Effluent NO ₂ ⁻ -N (mg/L)	0.72±0.27	0.93±0.24	0.84±0.29	0.87±0.34	0.91±0.17
Effluent NO ₃ ⁻ -N (mg/L)	2.20±0.42	3.39±0.26	3.87±0.05	3.84±0.06	3.75±0.12
Effluent PO ₄ ³⁻ (mg/L)	0.03±0.02	0.07±0.03	0.11±0.01	0.12±0.01	0.17±0.03

Note: Effluent of algal tank is admitted to the duckweed tank; hence, the effluent value reported for algal tank in the Table 3, for respective parameter, is the influent value for the duckweed tank for that parameter.

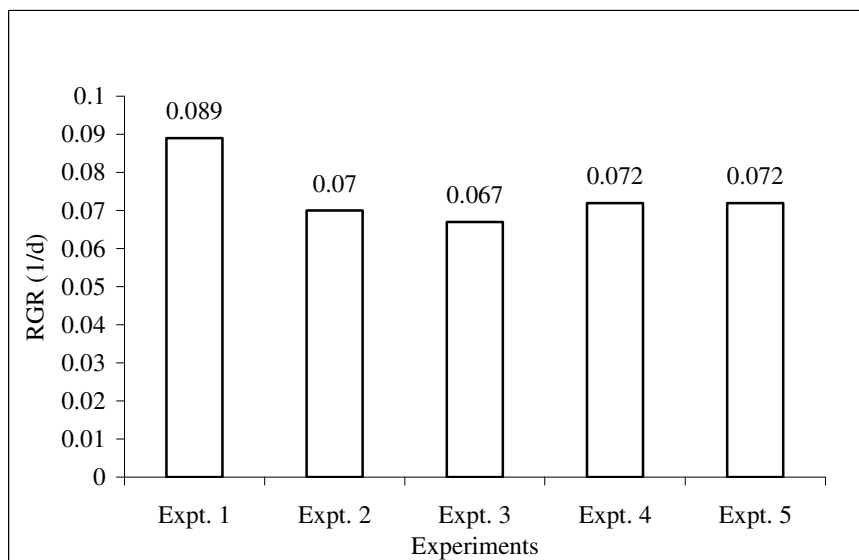


Fig. 3 Relative growth rate of *L. gibba* in different experiments

Table 5: Water quality parameters in fish culture tank

Parameter	Expt. 1	Expt. 2	Expt. 3	Expt.4	Expt. 5
pH	8.41 ± 0.20	7.82±0.42	8.05±0.27	8.46±0.20	8.37±0.11
DO (mg/L)	5.48 ± 0.32	5.057±0.84	5.28±0.54	5.10±0.37	5.36±0.47
Temperature (°C)	27.88±0.67	24.27±1.94	20.19±1.42	18.79±0.89	25.3±0.51
COD (mg/L)	32.4±4.6	34.9±1.7	34.4±2.1	29.6±5.2	53.1±3.6
BOD (mg/L)	16.4±1.8	20.8±2.2	21.4±1.9	21.3±2.3	24.3±3.4
TSS (mg/L)	14.7±3.9	16.5±2.5	24.2±2.5	18.3±2.5	14.9±1.5
NH ₄ ⁺ -N (mg/L)	0.76±0.27	1.61±0.11	1.82±0.22	1.71±0.19	1.70±0.21
NO ₂ ⁻ -N (mg/L)	0.69±0.24	1.37±0.27	1.43±0.15	1.33±0.25	0.95±0.16
NO ₃ ⁻ -N (mg/L)	2.19±0.44	3.69±0.42	4.02±0.17	3.87±0.12	3.77±0.18
PO ₄ (mg/L)	0.041±0.01	0.08±0.03	0.18±0.02	0.18±0.078	0.16±0.04

Table 6: Proximate composition of *S. platensis*

	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
Protein (%)	63.25	61.35	56.26	61.27	59.73
Fat (%)	6.21	7.36	8.46	7.61	7.42
Moisture content (%)	10.29	11.83	12.42	10.95	13.24
Ash (%)	11.46	15.63	15.34	13.67	13.82
Carbohydrate (%)	8.79	3.83	7.52	6.5	5.79

Table 7: Proximate composition of *L. gibba*

	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
Protein (%)	31.54	21.61	26.47	23.73	28.31
Fat (%)	4.21	8.52	6.46	7.13	7.27
Moisture content (%)	94.14	93.21	93.24	93.52	93.73
Ash (%)	11.63	10.32	11.26	10.83	10.13

CONCLUSIONS

The wastewater treatment and reuse through this integrated system of UASB reactor followed by algae and duckweed pond is simple in terms of operation and maintenance, and therefore suitable for eco-friendly disposal of wet-waste which are generated in kitchens of residential complexes and canteens of big motels, hotels, factories, etc. The UASB reactor demonstrated

higher COD and BOD removal efficiency at higher OLR; whereas, TSS removal efficiency was higher at lower OLR and higher HRT. The nutrient recovery from UASB reactor effluent can be achieved successfully by employing algal and duckweed tank for post treatment of UASB effluent. Having duckweed as a last treatment unit eliminates the appearance of algae in higher concentration in the

effluent and maintains desired water quality, required for aquaculture as demonstrated in this experiment.

The use of treated water, from this integrated system, for fish culture is successfully demonstrated in this study. During the treatment of wastewater by this integrated system, valuable by-products, like biogas from UASB reactor, algal and duckweed biomass from the respective algal and duckweed tanks were obtained, which can be used as protein supplement in feed for cattle, poultry and fishes.

Thus, by recovering these by-products and by practicing aquaculture in the treated water, the overall operation of wastewater treatment and aquaculture can be made self sustainable.

REFERENCES

- [1] Lettinga, G., A. DeMan, A.R.M. VanderLast, W. Wiegant, K. VanKnippenberg, J. Frijns, J.C.L. Van Buuren (1993) Anaerobic treatment of domestic sewage and wastewater. *Water Sci Technol*, 27(9): 67-73.
- [2] Van-Haandel, A.C., G. Lettinga (1994) *Anaerobic Sewage Treatment: A Practical Guide for Regions with a Hot Climate*. John Wiley and Sons, Chichester, England.
- [3] WHO (1989) Health guideline for use of wastewater in agriculture and aquaculture, WHO Tech. Rep. Ser., No. 778, WHO, Geneva: 32-41.
- [4] Shelef, G., Y. Azov, R. Moraine (1982) Nutrients removal and recovery in a two-stage high-rate algal wastewater treatment system. *Water Sci Technol*, 14: 87-100.
- [5] Dixo, N.G.H., M.P. Gambrill, P.F.C., Catunda, A.C. Van-Haandel (1995) Removal of pathogenic organisms from the effluent of an up-flow anaerobic digester using waste stabilization ponds. *Water Sci Technol*, 31(12): 275-284.
- [6] Oron, G., D. Porath, H. Jansen (1987) Performance of the duckweed species *Lemna gibba* on municipal wastewater for effluent renovation and protein production. *Biotechnol Bioeng*, 29: 258-268.
- [7] Skillicorn, P., W. Spira, W. Journey (1993) Duckweed aquaculture – A new aquatic farming system for developing countries. The International Bank for Reconstruction and Development, The World Bank, Washington, DC, USA.
- [8] Oron, G. (1994). Duckweed culture for wastewater renovation and biomass production. *Agric. Water Manage*, 26: 27-40.
- [9] Van der Steen, P., A. Brenner, G. Oron (1998) An integrated duckweed and algae pond system for nitrogen removal and renovation. *Water Sci Technol*, 38 (1): 335-343.
- [10] Rippka, R., J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology*, 111: 1-61.
- [11] APHA. (American Public Health Association, American Water Works Association, and Water Pollution Control Federation). (1989) *Standard Methods for the Examination of Water and Wastewater*. 17th edition. APHA. Washington, DC.
- [12] Kamilya, D., D. Sarkar, T.K. Maiti, S. Bandyopadhyay, B.C. Mal (2006) Growth and nutrient removal rates of *Spirulina platensis* and *Nostoc muscorum* in fish culture effluent: a laboratory-scale study. *Aquaculture Research*, 37: 1594-1597.
- [13] Hunt, R. (1978). *Plant Growth Analysis*. Edward Arnold, London, 67pp.
- [14] El-Gohary, F., S. El-Hawarry, S. Badr, Y. Rashed (1995) Wastewater treatment and reuse for fish aquaculture. *Water Sci Technol*, 32 (11): 127-136.
- [15] Sayed I.K.S., A.A.M. Fegala (1995) Two-stage UASB concept for treatment of domestic sewage including sludge stabilization process. *Water Sci Technol*, 12: 55-63.
- [16] Mario, T.K., A.J. Field, R. Kleerebezem, G. Lettinga (1994) Treatment of low strength soluble wastewaters in UASB reactors. *Journal of Fermentation and Bioengineering*, 77(6): 679-686.
- [17] Demirer, G.N., S. Chen (2005) Anaerobic digestion of dairy manure in a hybrid reactor with biogas recirculation, *World J. Microbiol. Biotechnol*, 211: 509-1514.
- [18] EL-Shafai, S.A., F.A. El-Gohary, F.A. Nasr, V.D.S. Peter, H.J. Gijzen (2007) Nutrient recovery from domestic wastewater using a UASB-duckweed ponds system. *Bioresource Technology*, 98: 798-807.
- [19] Michaud, S., N. Bernet, P. Buffiere, M. Roustan, R. Moletta (2002) Methane yield as a monitoring parameter for the start-up of anaerobic fixed film reactors. *Water Research*, 36: 1385-1391.
- [20] Parawira, W., M. Murtoa, R. Zvauryab, B. Mattiassona (2006) Comparative performance of a UASB reactor and an anaerobic packed-bed reactor when treating potato waste leachate. *Renewable Energy*, 31: 893-903.
- [21] Patnaik, S., A. Sarkar, A. Mitra (2001) Alginate immobilization of *Spirulina platensis* for wastewater treatment. *Indian journal of Experimental Biology*, 39: 824-826.
- [22] Lodi, A., L. Binaghi, C. Solisio, A. Converti, M. Del Borghi (2003) Nitrate and phosphate removal by *Spirulina platensis*. *Journal of Industrial Microbiology and Biotechnology*, 30: 656-660.
- [23] Zirschky, J., S.C. Reed (1988) The use of duckweed for wastewater treatment. *J. Wat. Poll. Cont. Fed.*, 60(7): 1253-1258.
- [24] Korner, S., J.E. Vermaat (1998) The relative importance of *Lemna gibba* L., bacteria and algae for nitrogen and phosphorous removal in duckweed-covered domestic wastewater. *Water Research*, 33(12): 3651-3661.
- [25] Gaigher, I.G., D. Porath, G. Granoth (1984) Evaluation of Duckweed (*Lemna gibba*) as Feed for Tilapia

(*Oreochromis Niloticus* X *O. Aureus*) In A Recirculating Unit. Aquaculture, 41: 235-244.

- [26] Becker, E.W., L.V. Venkataraman (1984) Production and utilization of the blue-green alga *Spirulina* in India. Biomass, 4(2): 105-125.
- [27] Boniardi, N., G. Vatta, R. Rota, G.A. Nano, S. Carra (1994) Removal of water pollutants by *Lemna gibba*. Chem. Eng. J., 54: 41-48.
- [28] Ennabili, A., M. Ater, M. Radoux (1998) Biomass production and NPK retention in macrophytes from wetlands of the Tingitan peninsula. Aquat. Bot., 62: 45-56.
- [29] Culley, D.D. Jr., E.A. Epps (1973) Use of duckweed for waste treatment and animal feed. J. Wat. Poll. Cont. Fed, 45 (2): 337-347.
- [30] Mahadevaswamy, M., V.L. Venkataraman (1986) Bioconversion of Poultry Droppings for Biogas and Algal Production. Agricultural Wastes, 18: 93-101.
- [31] Teasa, J., J.R. Hebertb, J.H. Fittonc, P.V. Zimbada (2004) Algae – a poor man's HAART?. Medical Hypotheses, 62: 507-510.